Intercellular Adhesion Molecule-3 (CD50) on Human Epidermal Langerhans Cells Participates in T-Cell Activation

Marcel B.M. Teunissen, Cock W. Koomen, and Jan D. Bos
Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Three different intercellular adhesion molecules (ICAMs) have been identified acting as ligand for counter-receptor leukocyte-function–associated antigen-1 (LFA-1) (CD11a/CD18). We have recently shown that ICAM-1 (CD54) is present on cultured human epidermal Langerhans cells but not on freshly isolated Langerhans cells, and that this molecule participates in the generation of an antigen-specific T-cell response. ICAM-2 (CD102) was not involved because this molecule is expressed by neither fresh nor cultured Langerhans cells. In this study, the presence of ICAM-3 (CD50) on Langerhans cells was examined. Flow cytofluorometric analysis demonstrated that ICAM-3 is strongly displayed by fresh Langerhans cells, and daily determinations showed that the level of this trypsin-resistant molecule remained nearly unchanged during *in vitro* culture for up to 4 d, indicating that Langerhans cells constitutively express this molecule. Analysis of RNA extracted from purified cultured Langerhans cells by means of reverse transcriptase-polymerase chain reaction demonstrated the presence of mRNA specific for ICAM-3. Antigen-specific T-cell responses triggered by Langerhans cells were dose-dependently inhibited by anti–ICAM-3 if the antibody was added within the first 16 h of T-cell stimulation. Simultaneous addition of anti–ICAM-1 and anti–ICAM-3 synergistically inhibited T-cell responses, although a total block was never achieved. Pretreatment of Langerhans cells with anti–ICAM-3 resulted in a reduced T-cell response, whereas pretreatment of T cells did not. These results suggest that ICAM-3 on Langerhans cells, like ICAM-1, is functionally involved in the initiation of antigen-specific activation of T cells, but the expression of these two ICAMs on Langerhans cells is differently regulated. ***Key words: ICAM-1/antigen presentation. J Invest Dermatol 104:995–998, 1995***

Interaction between the antigen/major histocompatibility complex displayed by the antigen-presenting cell (APC) and the T-cell receptor induces signal transduction, resulting in antigen-specific activation of the T cell. However, optimal T-cell stimulation also requires antigen-independent costimulation mediated *via* interaction between so-called adhesion molecules on APC and their ligands on T cells [1–3]. Epidermal Langerhans cells belong to the family of dendritic cells, which are specialized, powerful APC for the induction of T-cell responses [4,5]. Intercellular adhesion molecule (ICAM-1) (CD54), leukocyte-function–associated antigen (LFA)-3 (CD58), and B7/BB1 (CD80), absent on freshly isolated human Langerhans cells but clearly present on cultured Langerhans cells, have been documented to play a role in T-cell activation [6–9]. ICAM-2 (CD102), a second ligand for LFA-1 (CD11a/CD18), did not participate because this molecule was not expressed by freshly isolated Langerhans cells or cultured Langerhans cells [9]. Recently, ICAM-3 (CD50) was defined as the third receptor for LFA-1, differing from ICAM-1 and ICAM-2 in cDNA and amino acid sequences and in cellular distribution [10–12]. Although the presence of ICAM-3 on Langerhans cells *in situ* has been demonstrated [13], it is not yet clear whether ICAM-3 on Langerhans cells plays a significant role in the activation of T cells. In this study, we demonstrated that both freshly isolated Langerhans cells and cultured Langerhans cells exhibit a comparable strong expression of ICAM-3 and show that this molecule on Langerhans cells is functionally involved in the initiation phase of antigen-specific T-cell proliferation.

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Reprint requests to: Dr. Marcel B.M. Teunissen, Department of Dermatology, K2-209, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands.

Abbreviations: APC, antigen-presenting cell(s); LFA-1, leukocyte-function–associated antigen-1.

MATERIALS AND METHODS

Antibodies and Reagents Five different anti–ICAM-3 monoclonal antibodies (MoAbs) were used in this study: hybridoma supernatants HP2/19, TP1/24, and TP1/25 (all from F. Sánchez, Madrid [13]) and purified proteins of CBR-IC3/1 (A.R. de Fougerolles [10]) and BRIC79 (D.J. Anstee; International Blood Group Reference Laboratory, Bristol, UK [14]). All other primary and secondary antibodies have been described [9]. Cells were cultured in Iscove's modified Dulbecco's medium (Gibco, Paisley, Scotland) supplemented with 10% pooled normal human serum and 50 μg/ml gentamicin. *Candida albicans* antigen was obtained from ARTU Biochemicals (Lelystad, The Netherlands).

Isolation of Cells Langerhans cells were isolated as described in detail before [9,15]. In brief, a single epidermal cell suspension was obtained after trypsinization (0.25% trypsin, 30 min, 37°C) of normal human breast or abdominal skin. After discontinuous Ficoll-Metrizoate density gradient centrifugation, cells between densities of 1.070 and 1.052 g/cm^3 were collected. This cell population consisted of 50% to 80% CD1a$^+$ cells. T cells

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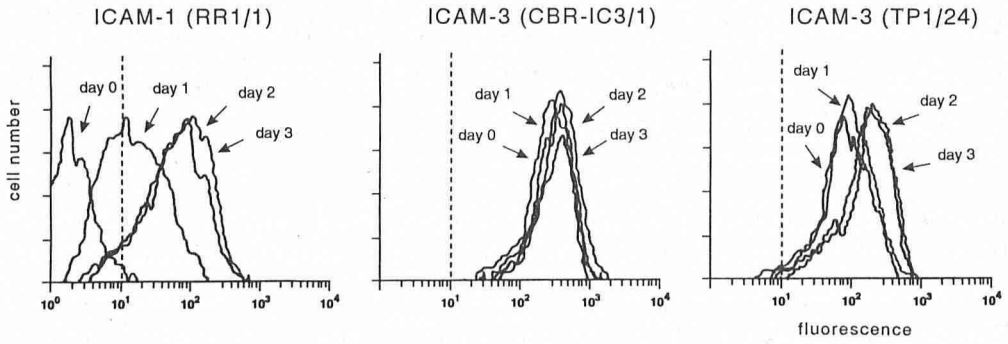

Figure 1. Both fresh and cultured human Langerhans cells express ICAM-3. Langerhans-cell–enriched suspensions were double stained and gated for cells positive for HLA-DR–fluorescein isothiocyanate (green fluorescence). Gated cells were analyzed for the presence of ICAM-1 or ICAM-3 (red fluorescence; shown in this figure). Langerhans cells were tested directly after isolation (day 0) or at indicated times after isolation. The level of these ICAMs at day 4 (not shown) was identical to that at day 3. These data are representative of three independent experiments.

were purified as described previously [9]. Peripheral blood cells were subjected to Lymphoprep (Nycomed, Oslo, Norway) and discontinuous Percoll (Pharmacia, Uppsala, Sweden) density gradient centrifugation, followed by E-rosetting of the high-density cells and plastic adherence. The population of rosetted nonadherent cells contained more than 93% $CD3^+$ cells. Low-density peripheral blood cells that were plastic adherent were used as a source of monocytes (more than 90% $CD14^+$).

Fluorescence-Activated Cell Sorter Analysis Cells were double stained by successive incubations with primary MoAb, phycoerythrin-conjugated (red) goat anti-mouse Ig, and fluorescein-isothlocyanate–conjugated (green) anti–human leukocyte antigen (HLA)-DR [9]. Fluorescence intensity was determined by a FACStar (Becton Dickinson, Mountain View, CA).

Reverse Transcriptase-Polymerase Chain Reaction (PCR) Langerhans-cell–enriched cells were cultured overnight, centrifuged on a Nycodenz density (1.080 g/cm^3; Nycomed) gradient, and purified (almost 100%) by the so-called panning technique [7]. While the cells were still attached to the petri dish, 0.5 ml Trizol (Gibco) was added and total RNA was isolated according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using MLV-RT (Gibco) in a 20-μl reaction; 2 μl was taken for each PCR amplification. Trizol was also used to isolate the RNA from monocytes stimulated overnight with 10 μg/ml lipopolysaccharide. The PCR mix contained 50 ng of each primer per 50 μl, 250 μM dNTP mix (Pharmacia), 50 mM KCl, 10 mM Tris-HCl pH 8.1, 1.5 mM $MgCl_2$, 0.001% gelatin, and 1.25 units Taq polymerase (Gibco), and was covered with 20 μl mineral oil. Reactions were performed in 50 μl per well in microtest plates (Becton Dickinson) in a PTC-100 thermal cycler (MJ Research Inc, Watertown, MA) using the following program of 35 cycles: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Twenty microliters of each reaction was loaded on 1.4% agarose gels in TBE buffer (0.09 M Tris-borate; 0.002 M ethylenediaminetetraacetic acid, pH 8.0), and PCR products were visualized by ethidium bromide staining. The primers used were as follows: ICAM-3 sense primer 5′-CGAGTTCTTGCACAGGAACA-3′ (nt 1150–1169), ICAM-3 antisense primer 5′-CCTGAAGACGTACATTAAGGCC-3′ (nt 1513–1534), ICAM-1 sense primer 5′-TAGCAGCCGCAGTCATAATG-3′ (nt 1517–1536), ICAM-1 antisense primer 5′-AGTCTTGCTCCTTCCTCTTGG-3′ (nt 1880–1900), LFA-1 sense primer 5′-CGGCCAAAGACATCATCC-3′ (nt 912–929), and LFA-1 antisense primer 5′-CATGTGCTGGTATCGAGGG-3′ (nt 1360–1378).

T-Cell Proliferation Assay T cells (5×10^4/well) with 2×10^3 γ-irradiated (3000 rad) autologous cultured Langerhans cells and 10 μg/ml *Candida albicans* antigen were cultured in a 96-well round-bottomed microtiter plate for 6 d, the last 18 h in the presence of [^{3}H]thymidine. Control cultures were T cells alone or T cells with either antigen or cultured Langerhans cells. Incorporation of the isotope was determined by liquid scintillation spectroscopy and expressed as mean cpm of triplicate cultures ± SD. Results were statistically evaluated by the unpaired Student t test.

RESULTS

ICAM-3 Is Constitutively Expressed by Human Langerhans Cells Fresh Langerhans cells as well as Langerhans cells cultured for 1, 2, 3, or 4 d were subjected to double fluorescence staining, using their bright staining for HLA-DR for identification. All Langerhans cells displayed a clear expression of ICAM-3 at all time points tested and showed either no kinetics (CBR-IC3/1 and HP2/19) or a slight increase (TP1/24 and TP1/25) during culture **(Fig 1)**. The latter two MoAbs recognize a different domain on ICAM-3 than the former two MoAbs [16]. ICAM-3 was not present on HLA-DR–negative cells contaminating the Langerhans cell suspension. The constitutive expression of ICAM-3 on Langerhans cells contrasted with the dynamic expression of ICAM-1, which is absent on freshly isolated Langerhans cells and markedly up-regulated upon culture **(Fig 1)**. Because trypsin exposure is necessary to isolate Langerhans cells, the effect of this proteolytic enzyme on ICAM-3 was tested. ICAM-3 appeared to be fairly resistant to trypsinization, whereas ICAM-1 disappeared (n = 3; data not shown). Reverse transcriptase-PCR analysis of RNA extracted from overnight cultured Langerhans cells showed a clear signal specific for ICAM-3 **Fig 2)**, confirming the expression of this molecule at the mRNA level. Further, Langerhans-cell–derived RNA contained mRNA for ICAM-1 but lacked mRNA for LFA-1, which correlates nicely with earlier observations done by fluorescence-activated cell sorter analysis [9].

ICAM-3 on Human Langerhans Cells Is Involved in T-Cell Activation To determine whether ICAM-3 participates in antigen-specific T-cell activation triggered by Langerhans cells, we added MoAb against this molecule to a co-culture of T cells with Langerhans cells and antigen to block the function of ICAM-3. MoAbs against ICAM-1 and ICAM-2 were included as inhibitory and noninhibitory isotype-matched controls, respectively [9]. Anti–ICAM-3 caused inhibition of the T-cell response, indicating that ICAM-3, like ICAM-1, is involved in this response **(Fig 3)**. Addition of serial dilutions of the MoAb revealed that the inhibition was dose dependent (n = 2; data not shown).

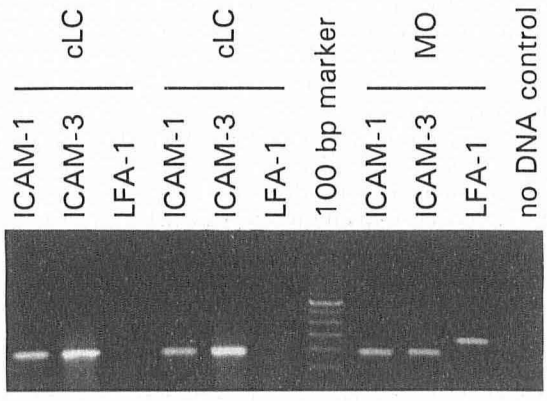

Figure 2. ICAM-1– and ICAM-3–specific mRNA is expressed in human Langerhans cells. Total RNA extracted from Langerhans cells derived from breast (*lanes 1–3*) or abdomen of a different donor (*lanes 4–6*) was subjected to reverse transcriptase-PCR, applying specific primers for ICAM-1, ICAM-3, or LFA-1. RNA from monocytes (MO; *lanes 8–10*) was used as a positive control. *Lane* 7, 100-kb marker; *lane 11*, PCR run in the presence of ICAM-3 primers but in the absence of cDNA. These results are representative of four separate experiments using different donors each time. cLC, cultured Langerhans cells.

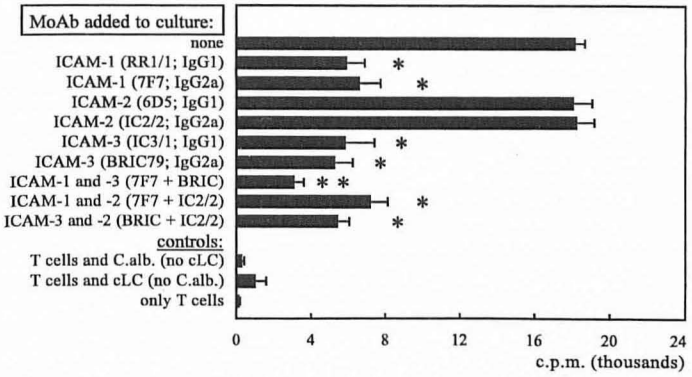

Figure 3. ICAM-3 is involved in antigen-specific T-cell stimulation by cultured Langerhans cells. T cells were stimulated with cultured Langerhans cells and *Candida albicans* (C. alb.) antigen in the presence or absence of the indicated MoAbs (10 μg/ml). Data are representative of three separate experiments and are expressed as mean cpm ± SD of triplicate cultures. $^{*}p < 0.05$; $^{**}p < 0.05$ as compared with the marked group (*). cLC, cultured Langerhans cells.

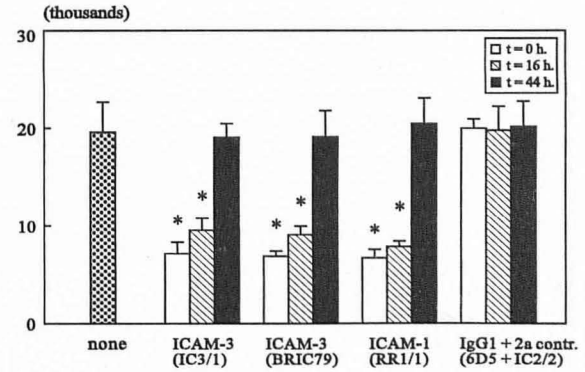

Figure 5. ICAM-3 on Langerhans cells has a functional role in the initiation of T-cell activation. Indicated MoAbs (10 μg/ml) were added to co-cultures of T cells plus cultured Langerhans cells and *Candida albicans* antigen at the start of the experiment (*open bars*) or after 16 h (*shaded bars*) or 44 h (*solid bars*) of culture. *Dotted bar* is control co-culture without MoAb. These data are representative of two experiments and are expressed as mean cpm ± SD of triplicate cultures. $^{*}p < 0.05$.

Combination of MoAbs against ICAM-3 and ICAM-1 resulted in additive inhibition; however, a complete block of the T-cell response was never achieved **(Fig 3)**. Preincubation of cultured Langerhans cells with MoAb before co-culture revealed that anti–ICAM-3 treatment produced inhibition at the level of Langerhans cells and not at the T-cell level **(Fig 4)**. As shown in **Fig 5**, anti–ICAM-3 exerted its inhibitory effect only if added within the first 16 h of culture, suggesting that ICAM-3 on Langerhans cells functions during an early phase of T-cell activation. The same held true for ICAM-1, which is in agreement with previous observations [9].

DISCUSSION

We found that ICAM-3 is clearly and constitutively displayed by Langerhans cells *in vitro*, extending a recent observation that showed the presence of ICAM-3 on human Langerhans cells *in situ* [13]. The reason why the two domains of ICAM-3 differ in their expression kinetics is not known, but one can speculate that freshly isolated Langerhans cells and cultured Langerhans cells differently glycosylate this molecule affecting only one domain. The presence of mRNA for ICAM-3 in Langerhans-cell–derived RNA provided further proof for expression of this molecule by Langerhans cells. The presence of ICAM-1–specific and the absence of LFA-1–specific mRNA in Langerhans cells confirm earlier data at the protein level and support the opinion that Langerhans cells do not express LFA-1 [9,17,18]. In this respect, it is interesting to note that Langerhans cells differ markedly from dendritic cells from the blood, lung, or tonsil as concerns their adhesion-molecule phenotype. Fresh Langerhans cells and Langerhans cells *in situ* constitutively express only ICAM-3, whereas ICAM-1, LFA-3, and B7/BB1 appear on Langerhans cells during culture [6–9], reflecting a process that is generally referred to as maturation. Dendritic cells express all these molecules, as well as ICAM-2 and LFA-1 [19–24]. It is conceivable that the state of differentiation and/or the microenvironment determine which adhesion molecules are expressed by Langerhans cells and dendritic cells. One may speculate further that the regulation of this adhesive makeup of Langerhans cells and dendritic cells is directly correlated to their functional properties at different sites (e.g., antigen presentation or migration).

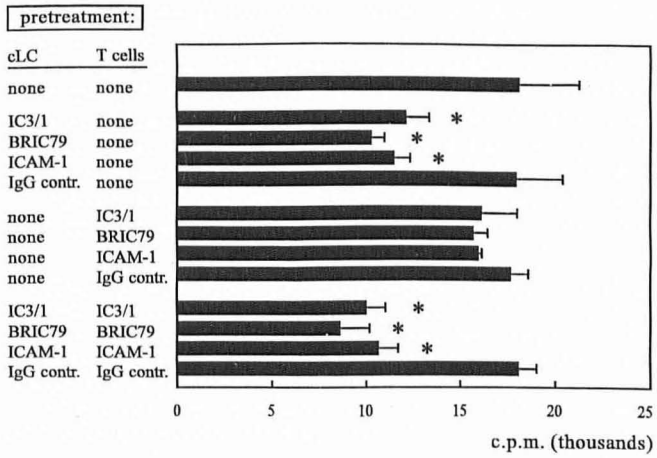

Figure 4. Pretreatment of Langerhans cells with anti-ICAM-3 inhibits antigen-specific T-cell stimulation. T cells, cultured Langerhans cells (cLC), or both were preincubated with the indicated MoAbs (10 μg/ml), washed carefully, and used in the antigen-specific T-cell proliferation assay. Results are representative of two separate experiments and are expressed as mean cpm ± SD of triplicate cultures. $^{*}p < 0.05$.

Addition of anti–ICAM-3 to co-cultures of T cells and Langerhans cells, as well as pretreatment of Langerhans cells with this MoAb to interfere with the function of ICAM-3 on Langerhans cells, inhibited the T-cell proliferation, indicating that ICAM-3 on Langerhans cells is functionally involved in the specific T-cell response to soluble antigen. The presence of anti–ICAM-3 MoAb during culture inhibited the T-cell response to a greater degree than pretreatment of Langerhans cells. Langerhans cells are probably able to re-express new ICAM-3 during the 6-d culture with T cells and, in the case of pretreatment, no interfering MoAbs were present, allowing the newly expressed ICAM-3 molecules to contribute to T-cell activation. Time-course studies revealed that ICAM-3 on Langerhans cells is functionally important during the initiation phase of T-cell activation, as was demonstrated earlier for ICAM-1 and LFA-3 [9]. We were not able to block completely the T-cell response with anti–ICAM-3. Even when MoAbs against ICAM-1 and ICAM-3 were applied simultaneously, no total block could be achieved, although some synergism was found. This may be explained by the fact that Langerhans cells are equipped with other adhesion molecules, such as LFA-3 and B7/BB1, providing alternative pathways to interact with T cells.

In conclusion, ICAM-3 on Langerhans cells participates in T-cell activation, probably (like ICAM-1) by facilitating cell-cell contact and delivering costimulatory signals to the T cell *via* LFA-1. In addition (and unlike ICAM-1), ICAM-3 itself is able to transduce signals across the plasma membrane [25,26]; thus, binding of ICAM-3 to its ligand LFA-1 on the T cell may stimulate the Langerhans cells as well. As concerns freshly isolated Langerhans

cells or Langerhans cells *in situ*, ICAM-3 is the sole receptor for LFA-1. Concerning cultured Langerhans cells, however, it is not yet known which of the two ICAMs on cultured Langerhans cells is the predominant counter-structure for LFA-1 on T cells. In this respect, it is interesting to note that avidity of the ICAMs is dependent on the conformational state of LFA-1 [27] and therefore is related to the state of activation of T cells.

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